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ABSTRACT

This manual provides practical advice on the appropriation, conservation, and documentation of a marine biological reference collection, in response to needs expressed by Mediterranean Arab countries. A reference collection is defined as a working museum containing a series of specimens with which biologists are able to compare their own material. The manual first reviews the principal methods for collecting marine biological samples from plankton, nekton, and benthos. Next, advice and recommendations are provided on the treatment of samples immediately after their collection and subsequently in the collection center. Topics considered include sorting, fixation and preservation, and labelling. The documentation which must accompany each sample throughout its existence is emphasized since, without such documentation, the sample has no scientific value. The last section of the manual touches on the problems of ethics and of the security of access to the collections by potential users. Additional information provided in appendices includes bibliographic references, a suggested classification scheme for the collections, addresses of suppliers of materials and equipment, examples of labels, and a schema explaining the principles of an adequate documentation system. (Author/JN)

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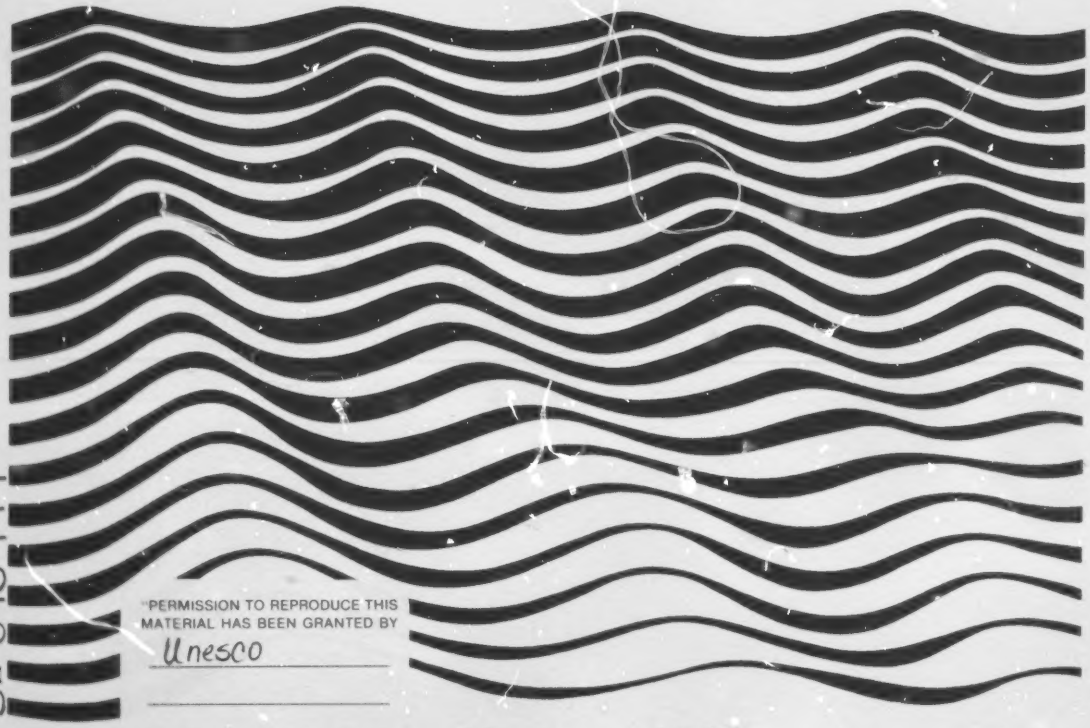
# Guidelines for marine biological reference collections



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# Guidelines for marine biological reference collections

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in response to a recommendation
by a Meeting of Experts from
the Mediterranean Arab countries

by J.C. Hureau and A.L. Rice

Unesco, 1983

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## PREFACE

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SUMMARY

The objective of Unesco and of the authors of this volume has been to furnish practical advice on the appropriation, conservation and documentation of a marine biological reference collection, in response to the needs expressed by the Mediterranean Arab countries. A reference collection is here defined as a working museum containing a series of specimens with which biologists are able to compare their own material. The manual first reviews the principal methods for the collection of marine biological samples from the plankton, nekton and benthos. Next, advice and recommendations are provided on the treatment of the samples immediately after their collection and subsequently in the collection centre, covering the topics of sorting, fixation and preservation, and labelling. The authors particularly emphasize the documentation which must accompany each sample throughout its existence. This is of prime importance, for without complete documentation a sample has no scientific value. The last section of the manual touches on the problems of ethics and of the security of access to the collections by potential users. Finally, the annexes provide the reader with very practical information: bibliographic references, a suggested classification scheme for the collections, addresses of suppliers of materials and equipment, examples of labels, and a schema explaining the principles of an adequate documentation system.

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## I. GENERAL INTRODUCTION

All ecological studies, whether for academic, commercial or environmental protection purposes, depend primarily upon the accurate identification of the organisms concerned. Such identifications can frequently be made using only the published literature, but for two main reasons this may not be possible. First, the relevant literature may not be locally available; few groups are covered by comprehensive up-to-date reviews and the literature may be scattered amongst many journals and books which are expensive, difficult, or even impossible to obtain. Second, even when the literature is available, the published descriptions may not give sufficient details to enable definitive identifications to be made or they may not deal with the relevant sex, size or developmental stage being considered. Under these circumstances, it is essential that the "unknown" specimens should be compared with previously identified reference material. In the ultimate case, comparison should be made with the unique "type" specimen on which, in modern biological nomenclature, each specific name is based. For historical reasons, most type specimens are lodged in one or another of the major museums in Europe or North America, and the regions from which they were obtained often not only lack the types, but do not have adequate collections of identified specimens of their local flora and fauna. Such reference collections are essential aids to the local ecologist whether he is concerned with the terrestrial, fresh water or marine environment.

This manual deals only with marine organisms, and since it resulted directly from a recommendation made at a meeting held under the auspices of Unesco in Tunis during September 1978 to discuss marine biological reference collections in the Mediterranean Arab countries, it is written specifically with the needs of these countries in mind (see Unesco reports in Marine Science, No. 6). Nevertheless, much of what it contains, and particularly the general information on fixation, preservation and curation, are equally applicable to other environments and other regions.

Thus, as considered here, a reference collection may be simply defined as a working museum containing a series of specimens which act as standards against which biologists may compare their own material in order to confirm determinations. To fulfil this function, the reference collection must be readily accessible to taxonomic specialists on the one hand, and to the ecologist on the other. The establishment and operation of such a reference collection involves a number of interrelated tasks several of which may be undertaken simultaneously. For simplicity, however, they are dealt with here separately under the following headings: collection, sorting, fixation and preservation, identification, storage, documentation and routine maintenance.

## II. SAMPLE COLLECTION

The literature on collection methods for marine organisms is enormous since almost every field marine biologist sooner or later develops his own collecting equipment or techniques, even if these are simply modifications of those used by previous workers. For collecting particular groups of organisms or for obtaining strictly quantitative samples, very specialized and sophisticated equipment is often necessary. Such equipment would be inappropriate for a reference collection centre and only relatively simple and inexpensive gears are therefore dealt with here. Most, if not all, of these gears can be purchased from commercial suppliers (see Appendix V), but many of them can also be made quite easily using locally available materials and expertise.

A. Plankton

Plankton collection methods have been described in Tranter (1968) and for more detailed information, we refer the users to this document.

The term plankton refers to those organisms living freely in the water column which are unable to maintain their position against the water currents but are carried along with them. Planktonic organisms are therefore generally rather small, ranging in length from a few microns to a centimetre or so, but the plankton also includes some quite large forms such as salps and jelly-fishes which may have dimensions of a metre or more.

Almost all plankton collecting methods are based on filtering the organisms from the water by passing it through a meshwork or net. Usually the net is towed behind a boat, but where a sufficiently strong current passes a jetty or promontory it may be possible simply to stream the net.

The simplest and most widely used plankton collector is the standard plankton townet (Figure 1) which consists of a conical net attached at its wider end to a canvas collar supported by a circular metal ring ranging from about 30 cm to a metre or more in diameter. A second canvas collar at the narrower end can be tied or clamped over a glass, metal or fibre-glass collecting bucket. The ring is attached to three rope bridles which in turn are connected to the towing warp, which extends a few metres beyond the net to end in a weight or a depressor to counteract the tendency of the net to lift during towing. A modification of this simple type of townet, the Hensen net (Figure 2), has a canvas collar in front of the main ring which restricts the size of the aperture, reducing the volume of water flowing into the net and therefore increasing the efficiency of filtration. Such nets are usually towed horizontally, but vertical or oblique hauls can also be made.

The preferred netting for townets, particularly in hot and humid environments, is now nylon monofilament since this material is more resistant to rotting than the bolting silk traditionally used in the past. The finer the netting, of course, the smaller will be the organisms retained by it. But there is no single mesh size suitable for collecting the whole size range of plankton, for the finer the mesh the greater will be the resistance to its passage through the water and its tendency to clog and therefore to filter inefficiently. A series of nets with different mesh sizes is therefore necessary and for the purposes of a reference collection three such nets should suffice, a coarse one (mesh aperture about 1.0 mm), a medium one (mesh aperture about 0.2 mm) and a fine one (mesh aperture about 0.55 mm). Since none of these nets will filter totally efficiently, each will retain organisms which should theoretically pass through the mesh so that the fine net will capture some examples of the very smallest planktonic organisms including protozoa and diatoms down to a few microns in diameter. However, to sample adequately this smallest constituent of the planktonic community, it is better to filter on board ship water collected by pump or water-bottle rather than to use a towed net.

All plankton nets must be towed at relatively low speeds (no more than 1.5-2 knots) if they are to filter efficiently and not be damaged. Very small nets, with mouth diameters of 35 cm or less, can be towed effectively at higher speed, though with some difficulty, from a rowing boat. Most sampling will, however, be undertaken with powered vessels and for these we recommend nets with a mouth diameter of about 0.5 m or so. Whatever net size is used, however, the length from the ring to the cod-end should be at least five times the mouth diameter.

Simple townets of the type described here will not provide quantitative samples for biomass studies but are quite adequate for the qualitative samples required for a reference collection, particularly in the upper few metres of the water column. However, when towed horizontally, they cannot be used to determine the vertical distribution of the plankton in the water column since the precise depth of fishing will be unknown, and in many cases the sample will be contaminated by organisms collected during the net's descent to and ascent from the desired fishing depth. A rough idea of the fishing depth can be obtained from the length of towing warp out and the angle at which it leaves the ship, but for an accurate data some form of depth recorder is necessary. Similarly, the effect of contamination on a sample can be reduced by increasing the length of a horizontal haul in relation to the descent and ascent time, or by making vertical hauls from successively greater depths. Unfortunately, the former technique tends to result in very large samples with an increased probability of clogging, while the latter often produces rather small and unrepresentative samples. The solution of both of these problems is the use of opening and closing mechanisms on the townets. Many such mechanisms have been devised, often involving quite complex engineering and electronics, but while some quite effective systems are relatively simple, they all involve a considerably more complex arrangement than described here. They are therefore beyond the scope of this manual, but the relevant literature is readily traceable through the key references given in the bibliography (Tranter, 1968, Wiebe et al., 1976 and Clarke, 1977).

B. Nekton

The nekton consists of those mid-water animals which, unlike the plankton, are able to swim against the prevailing currents. Generally, nektonic organisms are much larger than planktonic ones and the nekton includes most fishes, squids and all the marine mammals. To obtain many of these animals, a reference collection centre would be well advised to use locally available commercial gears such as traps, pelagic otter-trawls, seines and gill-nets (see FAO Catalogue of Fishing Gear Designs, FAO 1972), or to obtain samples from the local fishermen. But the division between the nekton and the plankton is not a clear-cut one and there are a number of animal groups such as the smaller fishes and squids, and some of the crustaceans such as the natant decapods and euphausiids, which are too small to be sampled adequately by commercial gears and yet are too fast-moving to be collected efficiently by conventional plankton nets.

The most widely used gear for sampling this micronectonic community (within the size range of about 2.0 to 10.0 cm) is the Isaacs-Kidd mid-water trawl. This trawl (Figure 3) consists of a conical net with a rectangular mouth fitted with a V-shaped vane along its lower edge which acts as a depressor to prevent the net from rising. Various sizes of trawls have been used successfully with mouth widths ranging from one to three or more metres, but for general use, a net with a mouth width of about two metres and a total length of some 8-9 metres is recommended. Details of the nets and rigging methods can be found in Aron (1962) and Isaacs and Kidd (1953).

A range of mesh sizes have also been used in Isaacs-Kidd mid-water trawls, including a series of different meshes decreasing in size from the mouth to the cod-end. But a good compromise mesh, recommended for all quantitative micronekton collection by the joint Working Group set up by SCOR, Unesco and ICES (Tranter, 1968), is one of 12.5 mm stretch mesh nylon throughout, supported by an outer coarse mesh of about 6.5 cm knot to knot. The condition of the catch is greatly improved by the inclusion of a short fine-meshed section of 3 mm mesh at the cod-end. Such a net should be fished at a ship's speed of about 3 knots.

C. Benthos

The benthos consists of all those animals and plants which live in, on or closely associated with the sea-bed ranging from the inter-tidal zone to the bottoms of deep ocean trenches. In the Mediterranean, the tidal rise and fall is so restricted that the inter-tidal zone is rarely very extensive. Nevertheless, some sublittoral organisms occur in this zone while others are more or less restricted to it so that shore collecting can be extremely rewarding.

On rocky shores, the larger non-sessile animals can be collected by hand, with blunt forceps or finer paint brush. Sessile organisms must be carefully levered off with a thin-bladed knife to prevent damage or, where this is not possible, small pieces of rock should be broken off with the organism attached. Many inter-tidal animals either live permanently within rock crevices or retire into them while the tide is out. The motile forms can often be driven out of the crevices by squirting in a weak solution of formaldehyde or domestic bleach, but to obtain some forms it may be necessary to break open the crevice with a crowbar. Clumps of seaweed provide a protective covering for many species during low water and a permanent home for many more, both sessile and motile. Such clumps, and particularly the holdfasts, should therefore be inspected very carefully. The associated animals can often be driven from seaweeds by leaving them in a small quantity of sea water for some hours to allow the oxygen tension to become seriously reduced, or by plunging the weed into fresh water or very dilute formaldehyde.

Rock pools should be inspected particularly carefully since they often contain a rich fauna and flora. Many rock pool animals can be collected by hand but a fine-mesh hand-net and a wide-mouthed pipette may be useful for obtaining the fast-moving forms.

Soft inter-tidal sediments, such as gravels, sands and muds, usually have few forms living on the surface during low water. Under these circumstances the sediment must be dug with a fork or spade and sifted through sieves either in the field or in the laboratory. If a series of sieves is employed, ranging in mesh size from about 2.0 mm to 50 microns, the fauna will be separated into convenient size fractions for further sorting and most of the fauna will be retained. In the finest of these fractions the retained material will contain a good deal of sediment and dead organic material as well as the desired animals. This can be hand-sorted under a microscope, but although this is desirable, and indeed essential to obtain some components of the fauna, it is very time-consuming and one or more "short-cut" techniques may be employed. Several such short-cut techniques have been devised, all depending to a greater or lesser extent on differences in density between the sediment and the animals. An excellent summary has been produced by Birkett and McIntyre (1971) and this should be consulted for details of the different methods.

The direct hand-collecting methods discussed above for the inter-tidal zone can be extended subtidally by SCUBA diving to depths of about 50 m. Diving is particularly valuable in sampling on hard substrates where trawls, dredges and grabs are unsatisfactory. On soft sandy and muddy substrates, which generally predominate subtidally, and particularly at depths greater than those routinely reachable by divers, indirect sampling is inescapable.

As with mid-water sampling, no single gear is suitable for sampling all of the benthic organisms, from the very small and relatively immobile meiofauna to the very active fishes and crustaceans which may be several orders of magnitude larger.

For sampling at the smaller end of this range, grabs and corers are designed to penetrate the sea-bed to a varying extent and to retrieve a sample of the sediment and the associated fauna without in any way sieving it until it has been brought on board. A wide range of different corers and grabs have been designed using various methods of achieving penetration and closure. One of the simplest but nevertheless very effective and widely used grabs is the Petersen grab (see Holme, 1964) (Figure 4). This consists of two jaws which are hinged together and are held open while the wire is taut during lowering. When the grab reaches the bottom and the wire becomes slack a release mechanism operates so that when the grab is hauled in the two jaws are pulled together and take a bite from the sea-bed. A model taking a 0.2 $m^2$ sample is normally employed. A similar mechanism is employed in the van Veen grab (Figure 5) where, however, the leverage is improved by having long arms attached to the buckets.

In the Smith-McIntyre grab (Figure 6), the jaws are mounted within a metal frame which also carries two trigger plates. When these are raised by contact with the bottom, the jaws are closed by a powerful spring mechanism and closure is completed by tension on the cable when hauling begins. The Smith-McIntyre grab is relatively light, weighing only about 45 kg, and is therefore suitable for use from small ships.

All grabs tend to force themselves away from the sea-bed as the jaws close, particularly where the sediment is coarse or very firmly packed. To overcome this problem, and also to achieve greater penetration and less disturbed samples, a variety of corers have been designed. These consist of metal cylinders of various shapes and dimensions which are forced into the sea-bed by weights, springs or pistons and are then closed either before or during withdrawal. While several of these have been used successfully both in shallow water and in the deep-sea, they are generally too cumbersome and complicated for routine use by a reference collection centre. However, a small Reineck box corer has been developed for use from fairly small vessels (Figure 7). For further details of these and other benthic sampling devices, reference should be made to Holme and McIntyre (1971).

For sampling on relatively firm bottoms, and for obtaining larger samples than those obtained by grabs and corers, towed gears are available which scrape the sediment into a collecting net rather than attempting to obtain an undisturbed sample. Again, a wide range of dredges and trawls is available and the choice of which type is employed will depend largely on the degree of penetration to be achieved, the type of bottom to be sampled and the power of the ship.

The most widely used gear of this type is the naturalist's dredge (Figure 8) consisting of a rectangular mouth frame which carries the net and has two hinged towing arms, one attached to the towing warp and the other joined to the first with a weak link. The net used will depend upon the type of ground to be sampled, ranging from interlacing metal rings for very rocky bottoms to hand- or machine-made mesh of trawl twine with a mesh size of about 25 mm knot to knot. To retain sediment or the smaller organisms, an inner liner of fine mesh netting can be added. The naturalist's dredge is very simple to use and will fish either way up although it does not penetrate far into soft sediments and therefore does not sample the infaunal animals very effectively. A dredge with a mouth width of 30-40 cm can be fished and hand-hauled from a small boat, but larger models require power both during fishing and hauling.

For greater penetration into soft sediments, an anchor dredge is required (Figure 9). These are basically similar to the naturalist's dredge but are rather more heavily constructed and generally have the towing arms attached to the frame assymetrically to cause the dredge to dig into the sea-bed.

Naturalists' dredges and anchor dredges tend to fill up with sediment rather rapidly and there is therefore little point in making hauls with them lasting more than a few minutes. Consequently, they sample only a small area of the sea-bed and do not collect the rarer epibenthic organisms effectively. For these animals it is better to use a beam trawl or Agassiz trawl, designed to skim over the bottom and to retain the larger epibenthic organisms while allowing the smaller animals and sediment to escape.

The beam trawl (Figure 10), is still used commercially in many areas and has a mouth which is held open by a wooden beam, usually 2-5 m long, with iron runners at either end. The upper leading edge of the net is attached to the beam while the lower one is attached to a weighted chain which curves back behind the head rope. Mobile animals disturbed by the foot rope, or by additional "tickler" chains which may be fitted ahead of the net, cannot escape upwards because of the top of the net. In the Agassiz trawl, which is really a double-sided beam trawl designed to fish equally effectively either way up (Figure 11), both the foot rope and head rope are the same length so that his gear tends to catch fewer fishes and other highly mobile forms than does the beam trawl. For general collecting a beam or Agassiz trawl with a mouth width of 1.5-2.0 m should be used, fitted with a net of about 12.5 mm mesh, though a section of finer mesh, down to about 5 mm, may be fitted towards the cod-end to retain some sediment and smaller organisms. In shallow water, these trawls should be towed over the bottom at 2-3 knots and with a towing warp length equal to about 2.5-3 times the water depth. Hauls of 30 minutes or more may be made without too much clogging, but in any unexplored region short hauls should first be made since an unexpectedly large catch of sediment, or for that matter animals, may be very difficult to bring aboard.

For the largest epifauna, including many fishes, commercial otter trawls can be used. These do not have a rigid mouth frame, but are held open by wooden or steel planing surfaces, the otter boards. Otter trawls are used in a wide range of sizes from small ones with head rope lengths of a few metres and doors weighing only a few kilograms, used in some crustacean fisheries, to enormous trawls with head rope lengths measured in tens of metres and doors weighing a tonne or more. Rigging and working otter trawls is a complex business best left to a professional fisherman, but since such trawls are now very widely used it should be possible to obtain the services of a suitable expert locally.

## III. SAMPLE TREATMENT IN THE FIELD

Once collected, material for a reference collection should be sorted, killed, and fixed as soon as possible, particularly in the relatively warm conditions likely to be encountered in the Mediterranean countries where post-mortem deterioration is very rapid. Plankton collections can generally be fixed in their entirety immediately after capture, but benthic samples need some prefixation treatment firstly because it is essential to remove as much sediment as possible from the middle-sized and larger organisms, and second because some groups assume an almost unmanageably contracted or damaged state if they are plunged directly into fixative. However, no matter how much preliminary sorting or anaesthetization is undertaken, all material must be fixed within an hour or so after capture.

A. Preliminary sorting

We have assumed that the object of the reference collection sampling programme is qualitative rather than quantitative samples so that the division of the samples into different fractions is for convenience rather than for comparison with other sampling programmes.

Whatever the sampling gear, as soon as the sample comes aboard all the large and also the delicate organisms should be removed by hand and carefully washed in clean sea water. If the sample is very large and contains a considerable volume of sediment this process may be facilitated by washing it through a very coarse mesh of 5-8 mm. A large volume of sea-water will be required for this and the subsequent sorting procedures but it must be remembered that water pumped from the surface of the Mediterranean may have a temperature of 25°C or higher and this will cause rapid autolysis of any dead material. The large animals removed in this way should be further separated into their taxonomic groups, at least to phyla, and in many cases to lower taxa; a suggested taxonomic list for this first sorting is given in Appendix 1.

The fraction of the sample not retained on the coarse mesh and sorted in this way should now be passed through a series of stainless steel sieves of 2.0, 1.0 and 0.5 mm mesh, with finer meshes, down to 50µm, being used where meiofaunal samples are sought. Circular nesting sieves with stainless steel or brass frames are commercially available up to diameters of 45 cm, but if very large samples are to be dealt with larger sieves may have to be specially made using commercially available woven mesh or perforated sheet. This sorting process will again use large volumes of water, but it should be accomplished as gently as possible to minimize the mechanical damage to the sample. Having washed as much sediment as possible through each of these sieves the retained residues should be fixed as quickly as possible.

B. Killing, fixation and preservation

Since the taxonomy of different animal groups is based on different anatomical features which are not equally well preserved by any single procedure, special techniques have been developed for many taxa. As the reference collection develops, specialist taxonomists may ask for a particular procedure to be follwed in dealing with "their" group. Wherever possible, of course, such requests should be complied with. In this section, however, we outline a general procedure which should produce acceptable results for almost all groups.

1. Anaesthetization

Having roughly sorted and cleaned the catch where necessary, as described in the previous section, many of the animals, and the residues of benthic samples, can simply be placed into a suitable fixative (see below) in readiness for the subsequent sorting and final preservation. Many invertebrate groups, however, such as annelids, anemones and polyzoans, will contract if treated in this way, making subsequent examination and identification difficult. Other groups, such as many decapod crustaceans and echinoderms, will cast parts of their appendages if they are plunged suddenly into fixative. Pre-fixation anaesthetization is well worth attempting for such animals so that they can be preserved in a relaxed condition.

Many substances and tecnniques have been used successfully for anaesthetizing marine organisms, including simply chilling them, placing them in deoxygenated water, or slowly bubbling tobacco smoke through the water in which they are being held. Apart from these simple methods, one or other of the following anaesthetics should be suitable for most groups.

(a) Menthol, magnesium sulphate or chloral hydrate

After the animals have been allowed to expand in clean sea-water, crystals of one or other of the substances can be sprinkled on the surface of the water and allowed to dissolve. Alternatively, a 20-30 per cent solution of magnesium sulphate can be added gradually to the water, or the animals can be placed directly into a 2 per cent solution of chloral hydrate.

(b) Magnesium chloride

A 7.5 per cent solution of magnesium chloride ($MgCl_2,6H_2O$) is almost isotonic with sea-water and therefore does not result in osmotic damage to the tissues of marine animals. Animals to be anaesthetized can be placed directly into such a solution and then transferred to the fixative.

(c) MS 222-SANDOZ

A manufacturers code name for ethyl m-aminobenzoate which has been widely used for anaesthetizing invertebrates and cold-blooded vertebrates. Instructions on strengths and procedures to be used with different taxonomic groups are supplied by the manufacturers.

(d) Propylene phenoxetol

A good general anaesthetic for use with most invertebrate groups. When the animals are expanded, the propylene phenoxetol should be added so that it forms a globule on the bottom of the container not exceeding 1 per cent of the water volume. Anaesthetization may take from a few minutes to several hours, depending on the group.

2. Fixation

Fixation is the process of stabilizing the protein constituents of tissue after death so that they retain to some extent the form they possessed in life. Many substances have been used as fixatives, but none of them can be regarded as an all-purpose formulation since the choice will depend on the material to be fixed and the purpose for which it is to be used. However, for a reference collection which will not in general be used for histological or cytological studies, the best general fixative is undoubtedly formaldehyde or formalin.

Formalin is usually purchased as a 40 per cent aqueous solution of formaldehyde gas, together with various impurities. In making up dilutions, it is important to recognize the difference between percentages of formaldehyde and percentages of formalin. In discussing fixative strengths one normally refers to formalin, taking the commercial solution (40 per cent formaldehyde) as 100 per cent formalin. Material should be fixed in a 5-10 per cent formalin solution. Five per cent formalin is made up by diluting one part of the commercial solution with 19 parts of water. For marine organisms, sea-water should be used for this dilution to avoid distortion due to osmotic effects. Furthermore, since formaldehyde solutions are normally acid because of the presence of formic and other acids, the original undiluted formaldehyde solution should be buffered by an addition of 20 g of borax or 200 g of hexamine (hexamethyleretetramine) per litre of solution.

For simplicity, all collected material, whether benthic or planktonic, should be placed initially in 5 per cent (10 per cent for fishes) buffered formalin solution for fixation. An adequate volume of formalin is essential for satisfactory fixation. Ideally the volume of the formalin solution should be about ten times that of the sample; where very large samples are involved such an ideal ratio may be unattainable, but samples should never be fixed in less than three or four

When collected material is fixed, it must be transferred to a preservative liquid. This operation differs according to the zoological group and some calcareous organisms should be transferred to alcohol after about two days fixation in formalin (see paragraph IV, B).

C. Containers

Strong plastic tubs, with a capacity of 40 to 60 litres, are useful on board ship for the reception of the catch prior to the preliminary sorting.

Containers for the transport of specimens back to the laboratory should be easy to manipulate and robust. Polythene jars with screw tops and good seals are preferred, though glass containers may be used if sturdy cases are available with adequate dividers and packing to prevent breakages. A range of sizes from about 250 ml to 4 litres will normally be adequate, but for large benthic residues and for large individual specimens, such as fish, tubs with capacities up to about 60 litres may be needed (Figure 12). In all cases, narrow necks must be avoided since it may be difficult to introduce large specimens into such containers and impossible to remove them! If the journey back to the laboratory is likely to involve considerable disturbance, for instance by sea or over uneven roads, the containers must be filled as completely as possible with fixative, to reduce the possibility of mechanical damage.

D. Labelling and documentation

1. Labelling

It may sometimes be desirable to label samples externally by sticking or tying labels to the container or writing on the container itself. However, such external labels are very vulnerable to being rubbed or torn off or even to being eaten by rats, mice or insects! Whether external labels are used or not, therefore, internal labels are essential.

Such labels for immersion must be made of good quality 100 per cent rag paper, or a parchment, free from starch and mineral matter, and with a high wet strength. Matt finish polypropylene or polyethylene plastic labels may also be used, but these can only be written on with a soft lead pencil, whereas the most permanent technique is the use of a waterproof black India ink with paper labels. The ink must be allowed to dry thoroughly before the label is immersed.

For convenience and for standardization, it is desirable to have labels of a variety of sizes suitable for the different containers used to be pre-printed with spaces for the relevant information. Examples of such labels are given in Figure 13. The printing ink used for such labels must, of course, be waterproof as well as aldehyde, alcohol and glycol resistant.

2. Documentation

It is important that the relevant collection data should be written down at the time and not committed to memory for transcription at a later date. In general, such data should be included on the sample label, but it is sometimes more convenient to label samples simply with a numbered label, either a station number or an arbitrary code, which refers to the detailed data kept in a field notebook, or station sheet (Figure 14).

The minimum information to be recorded ultimately on the permanent label should be the date of collection, the locality, the depth and the type of net or collection method used. For collections obtained from a ship it is usually convenient to use unique station numbers on the label, recording the details of latitude, longitude, type of bottom and so on in a separate station log.

## IV. SAMPLE TREATMENT IN THE COLLECTION CENTRE

When a collection enters the Reference Collection Centre the material will have to be sorted to a greater or lesser extent, transferred from its temporary field containers into the more permanent "museum" containers, and also transferred from fixative into the final preservative. At this time the collection must be registered in the centre's documentation system.

### A. Sorting

Samples will arrive at the Reference Collection Centre in two main forms; first, the larger and more delicate organisms which were removed from the catch in the field and preserved separately, and second, the residues which may have have been separated into two or more size categories by sieving.

The separated specimens will probably need washing once more and will require some further sorting, but assuming that they have been fixed adequately they can be transferred to the final preservative to await identification. The residues, however, will require much more attention. Benthic samples will almost certainly require a good deal more washing, no matter how much attention they have received in the field. This must once more be carried out as gently as possible and with copious supplies of water, though fresh-water may be used this time. Remember that the washings from the coarse residues must be sieved through the finer meshes again to retain any small specimens which were not washed through the larger meshes during the initial sieving process.

Once the residues have been adequately washed they can be sorted into the categories given in Appendix 2, or into even smaller taxa if these can be recognized. For the comfort of the sorters, the residues can be retained in water at this stage since they will come to little harm as long as the sorting process takes only a few days and the samples were adequately fixed initially.

The residues must be sorted using techniques appropriate to their nature, ranging from the unaided eye for the coarser ones to binocular microscopes for the finer fractions. In all cases, however, the sorting procedure is easier and more efficient if only small quantities are dealt with at one time, forming a thin layer on the bottom of the sorting dish. Good illumination is also essential and both black and white sheets of plastic should be available so that the background can be varied to suit the sample.

### B. Preservation

The processes of fixation and preservation are often confused and, indeed, the distinction between the two is rather fine. Fixation is essentially a rapid process involving, among other things, the arrest of autolysis, that is the self-digestion of tissues by the contained enzymes. Once fixed, the preservation of biological material involves its long-term storage without serious distortion or other deterioration. Preservatives must therefore be bactericidal and fungicidal, but need not necessarily arrest autolysis. In practice, most preservatives (except the recently introduced "phenoxetols") do have some fixative action. Similarly, most fixatives, including formalin, are acceptable long-term preservatives. Although formalin is relatively cheap and non-flammable there are serious

disadvantages in its use as a preservative. First, material stored in it for a long time tends to become hard and brittle, making examination difficult and damaging. Second, even in buffered formalin the pH may change and cause decalcification. Finally, formalin is an unpleasant fluid to work with. For these reasons, most material after fixation should be transferred to a second fluid.

Unsorted plankton samples should be transferred after 7-10 days in formalin to Steedman's Solution (Unesco, 1976) made up as follows:

| | |
|---|---|
| Propylene phenoxetol | 0.5 ml |
| Propylene glycol | 4.5 ml |
| Buffered formaldehyde (commercial 40%) | 2.5 ml |
| Sea water | 92.5 ml |

The propylene phenoxetol should first be dissolved in the propylene glycol, then the buffered formaldehyde should be added, and finally the sea water. There is considerable disagreement amongst specialists about the most suitable pH for the preservation of various groups. The additon of 20 g of borax per litre of undiluted formalin, as recommended here, will hold the pH at about 8.0. It is important that the pH does not significantly exceed this level in order to avoid damage to crustaceans and jelly-like organisms.

All calcareous benthic organisms, such as foraminifera, most molluscs and the echinoderms, should be transferred to alcohol after about two days fixation in formalin. After fixation in formalin (10 per cent) for at least four days, fishes must be transferred to a weaker (5 per cent) solution of fresh formalin in which they can be left for several weeks; if the otoliths are to be examined, they must be removed before fixation. Non-calcareous groups, and particularly soft-bodied forms such as many coelenterates, polychaetes and ascidians, can be safely left in formalin and, indeed, this may be a better long term preservative than alcohol for some groups. Nevertheless, for simplicity of management and to reduce the chance of serious errors, we recommend that all benthic animals should be washed thoroughly in fresh water after fixation and transferred to 80 per cent ethyl alcohol.

Ethyl alcohol (ethanol) has been regarded for many years as the best general preservative for invertebrate animals, and is widely used in most of the old established national museum collections. In most countries pure ethanol is expensive, because of the high duty which it attracts. However, methylated spirit, which is a mixture of ethyl alcohol and methyl alcohol, is not normally subject to high duty and is quite adequate for routine preservation. Two broad categories of methylated spirit are usually available, mineralized and non-mineralized. Mineralized spirit, which contains a small amount of pyridine and in which the methyl component is crude methyl alcohol (wood naphta) is quite useless as a preservative since it forms a milky suspension when diluted with water. Non-mineralized spirit consists of 95 parts by volume of ethanol and five parts by volume of pure methyl alcohol. The grade of non-mineralized spirit called Industrial Methylated Spirit (IMS) is quite suitable for preserving biological material. It should be diluted to 80 per cent by volume with fresh water, that is four parts of IMS with one part of water. It is important to remember that alcohol extracts water from specimens placed in it, and, since animal tissues is made up largely of water, if the volume of the alcohol does not considerably exceed that of the material placed in it, its strength will eventually be reduced very considerably. The final strength of the alcohol in which the animals are preserved must be at least 50 per cent, so that starting with 80 per cent alcohol at least twice the volume of the specimens should be used for preservation or the alcohol should be changed after about one week.

Direct transference of soft-bodied animals from formalin into strong alcohol is apt to cause shrinkage. This can be avoided by placing them for a few hours successively in 30 per cent and 50 per cent alcohol before finally transferring them to 80 per cent alcohol for permanent storage. Fishes must be washed in running fresh water for 12 to 24 hours and then transferred to ethyl alcohol (70%).

C. Containers

The requirements of containers for the long-term storage of the collections are different from those used in the field. In the collection centre the containers will not generally be exposed to the same risks of breakage so that they do not need to be so robust. Nor will they need to be opened and closed quickly or with wet or cold hands. On the other hand, the specimens should, wherever possible, be easily visible through the walls of the containers and the lids should be completely air-tight to prevent drying-out through evaporation.

Traditionally, museum collections have generally been stored in glass bottles with individually made ground-glass stoppers which could be sealed with a smear of vaseline. While such containers are certainly very good, they may become very difficult to open after long-term storage. Moreover, they are now extremely expensive and very difficult to obtain. Two acceptable alternatives are available: glass jars with glass clip-on lids and rubber seals and glass jars with polypropylene snap-on lids, the former being preferred for long-term storage (Figure 12). If jars with rubber seals are used thin sheets of polythene must be inserted beneath the seals since they will otherwise be attacked and made ineffective by the preserving fluid.

These types of jars are available in a range of sizes but those with capacities of about 100 ml, 500 ml, 1,000 ml, 2,000 ml, and 3,000 ml should cover most requirements of the reference collection. Specimens which would be "lost" in the smallest of these jars should be placed in glass specimen tubes, closed with plugs of good quality tissue paper, and immersed in preserving fluid in a jar. To conserve shelf space large numbers of such tubes can be stored in a single large jar. Very large jars with lids in the form of flat glass discs are also available (see Appendix 5); these must be sealed with mastic.

Specimens or collections which are too large to be kept in the largest jars should be stored in polythene tubs: these are not sufficiently sealed to prevent evaporation of alcohol, and must therefore be used only to contain formalin.

D. Registration and labelling

As soon as the material is taken back to the collection centre, it must be registered in a registration book where the following information is written: name of collection (cruise or name of collector), zoological group (phylum, class, family, genus or species level), date of collection, locality (or latitude and longitude), depth, type of bottom or habitat, method of capture (type of net, line ...) and so on. Each specimen or group of specimens of the same taxonomic group should be given its own registration number.

The specimens must be permanently labelled. In general, the field labels should never be destroyed and replaced by new ones. Even when material is re-identified, the old label should be left in the container with the specimen since there is always a risk of mistakes being made when information is transcribed. However, permanent written labels must be made and remember that for these labels, pencils and ball point pens are useless. A good waterproof Indian ink must be used. A good alternative to written labels is the use of perforated labels on which the information (for example: the registration number) is punched mechanically. These perforated labels, using parchment or modern paper (such as

papertex or tyvek) can be kept for a very long time without any deterioration: this method is used in the Paris Museum and many existing labels were made 120 years ago.

As the material becomesidentified, whether at a high taxonomic level such as phylum or class, or at a much lower level such as genus or species, it is important that the name of the identifier or determinator (and the date of determination) should also be recorded on the label so that the reliability of the identification can be assessed by subsequent workers. This information should also be recorded in the registration book and on the filing cards (see following paragraph).

E. Documentation

The simplest and most versatile documentation system makes use of edge-punched filing cards which can carry a good deal of taxonomic and collection data (see Figure 15). For each container there will be a corresponding file card which carries the same information as the label and, in addition, details of the treatment received such as the duration of fixation, the nature of the final preservative, the type of container in which it is stored and the condition of the material. Finally, the card will carry a coded reference to the shelf position of the specimen or specimens within the collection. All of the cards referring to one station, locality, or collecting expedition should be filed together, whereas the samples themselves will be split up according to taxonomic groups. An alternative is to file both cards and samples according to a taxonomic order. The shelf position can be given by a composite number referring to the taxonomic position.

As the samples become more and more finely sorted these cards will be emended and new ones will be made out. If, for example, from the original sample a four-litre jar of decapod crustaceans was separated a card such as that shown in Figure 15 A would be produced. If, some time later, several galatheids and brachyurans were again separated from the original decapods, the first card would be emended and two new cards would be produced as in Figure 15 B. Finally, if the brachyurans were sent away to a specialist for identification and were returned identified as three distinct species the relevant card in Figure 15 B would be replaced by three specific cards as in Figure 15 C.

F. Arrangement of the collection

All shelves, cupboards and drawers in which specimens are stored should be clearly numbered and plans of the layout of the storage room or rooms should be displayed prominently near each entrance.

Unsorted collections, and newly-received collections awaiting registration and sorting, should be stored chronologically in a separate area kept specifically for this purpose and preferably adjacent to the curation room.

All sorted material should be stored according to a taxonomic hierarchical system (see Appendix 2), that is by phylum, class, order and family. The genera within each family and the species within each genus should be arranged alphabetically. Material identified only to a taxonomic level higher than the species should be stored together at the end of the space allotted to that taxon. Material identified only as "Crustacea", for example, should be stored at the end of the crustacean section, while material identified as "Decapoda" should be stored at the end of the section allotted to this order, and so on. Where material has been sent out on loan, its place on the shelf should be marked by a card carrying details of its whereabouts.

Jars, tubs and other containers should never be closely packed on the shelves. Instead, they should be arranged in single rows from the front to the back of the shelf with ample space between the rows so that the rear jars can be removed easily without displacing those in front.

This type of hierarchical arrangement inevitably includes a good deal of unoccupied shelf space at any one time since room must be allowed within each taxon for the acceptance of additional material without the need for a major rearrangement of the collections each time. The initial allocation of space therefore requires a good deal of forethought taking into account both the extent of each taxon in terms of the number of species and the size of the individual specimens. The amount of space provided for the phylum Chaetognatha, for example, should be considerably less than that provided for the crustacean order Decapoda. Groups with a very wide range of sizes, such as fishes, may require special arrangements, so that large containers or tubs for the larger specimens can be stored on lower shelves or on the floor, while the smaller specimens can be stored in jars on the shelves above them.

The collection itself should act as an aid to identification, the user being able to wander around the shelves comparing his unidentified material with the identified specimens. Thus, while the collection should be stored as far as possible in the darkness, or at least never in direct sunlight, very good lighting should be provided so that material can be seen clearly, even at the back of the shelving.

Material removed from the storage area by users should always be replaced by the reference collection staff to ensure its correct relocation.

G. Routine maintenance

Material removed from the storage area for any reason, either for sorting, for examination by a user, or for dispatch to a specialist, should be carefully examined and the stage of preservation checked. Where necessary the preservative should be topped up or changed, the material transferred to a new container, or new or additional labels produced.

Much of the collection, however, may remain unused on the shelves for many years and for this reason a routine system of maintenance must be established. A strict rotation should be undertaken so that one section of the collection is examined and curated in this way each week or month, so that the whole collection is dealt with over a period of no more than two years. Any jar or other container which has lost a significant proportion of the preservative within this period must be suspected of having an ill-fitting lid allowing evaporation, or a fracture allowing leakage. Such containers must be replaced.

As well as dealing with the contents of the jars or tubs, the exteriors of the containers and the shelves themselves should be carefully cleaned at this time. As each section of the collection is examined and maintained in this way, a card carrying the date when the work was completed should be attached to the shelving, preferably in a simple frame provided for this purpose.

During the early life of a reference collection, when the total collection is very small, such a system of routine maintenance may seem unnecessary. However, it is essential that a routine is established from the start if the collection, or some parts of it, are not to be neglected as it grows.

V. LOANS AND AVAILABILITY TO USERS

A reference collection, like that of a museum, is of little value unless it is accessible to users. These will fall into two main categories, the taxonomic

specialists who will identify the material in the collection, and the biologists, often not taxonomists, who will wish to identify their own material by comparison with the reference collection. While both types of user will sometimes work in the Reference Collection Centre itself the specialist taxonomists, in particular, will often need to be sent material on loan.

The relationship of the Reference Collection Centre staff with such specialist borrowers will inevitably be somewhat ambivalent since, on the one hand they will often be dependent upon the taxonomists to provide the identifications which will enable the collection to fulfil its function, while on the other their primary responsibility is to the collection itself. They will therefore sometimes find themselves in the difficult position of trying to balance the need to retrieve loaned material so that it may take its rightful place on the collection's shelves, against the equally important need to have material identified. It is impossible to give any general guidance in this area since each case will be unique and will have to be judged on its own merits. One important point can, however, be made. Professional taxonomists can rarely, if ever, be expected to devote time and effort to the identification of material without some form of compensation. On occasion a straightforward commercial arrangement with a financial inducement may be appropriate, though this is rarely either desirable or necessary. Much more satisfactory is an arrangement by which the taxonomist is able to further his own studies as a result of his relationship with the Reference Collection Centre. The Centre must therefore be prepared to allow the specialist to use the loaned material in systematic studies or to provide him, in the form of a gift, with material for his personal or institutional collection.

Details of when material is sent out to specialists and when it is returned should be entered on the edge-punched card system described above. However, so that the loan situation can be regularly reviewed a separate index system dealing only with material sent out from the Reference Collection Centre should be created. This can be most simply based on a printed loan form such as those used by most of the major national museums (see Figure 16). These forms should be produced in triplicate, preferably in three different colours. When a loan is sent out, details of the material being loaned is entered on all three forms and one copy is retained at the centre as a record. The other two copies are sent to the borrower, one to be retained for his record and one to be signed and returned to the Centre as a receipt for the material.

If a standard loan period of one year is used, the two copies of the loan form held at the Centre can be filed together with those of all other loans made in the same month, though not necessarily in the same year. These will thus be 12 batches of loan forms, one for January, one for February, one for March and so on. At the beginning of each month the loan forms in that month's batch are examined and reminders sent to the borrowers that the loan period has now expired so that the loan must either be returned or extended. At the end of the month any loans which have not been either returned or extended are moved into the following month's batch so that they continue to be under review.

When a collection is very large and when the loans are numerous it may be useful to have a computer system to manage the collection. For more details on these modern and expensive systems, see McAllister (1978) and Gosse (1978).

## VI. SECURITY AND ETHICS

For a reference collection to be of real value to a user he must be able to accept the identifications with considerable confidence. It must be assumed that such confidence is well founded in the case of identifications made by specialist taxonomists. But many identifications will be undertaken by members of the reference collection staff who may not be specialists in the particular group. In

these cases it is essential not only that the identity, and therefore the status of the identifier is clearly documented, but also that any doubts about the accuracy of the determination are expressed. In this context it is much more valuable for an identifier to stop at a taxonomic level at which he is confident of his identification rather than to proceed beyond this level with uncertainty and therefore possibly mislead the user. There is no disgrace in being unable to identify a specimen, but there is in labelling one with unwarranted confidence.

The staff of a reference collection centre are custodians of the collection responsible for protecting it not only from the effects of natural deterioration but also from any harm due to human agency. It is thereforeimportant that all visitors to the collection should be treated with some care until they have demonstrated a responsible attitude towards the material. Until this is so they should not be allowed to wander unaccompanied through the collections, for a surprising amount of chaos, and work for the curatorial staff, can be created by an irresponsible visitor in a very short time. Similarly, a visitor examining material in the Reference Collection Centre should be reminded of the need for care in dealing both with the specimens and with the labels. Thereafter they should be carefully, if discreetly, kept under surveillance until it is obvious that they can be relied upon.

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Appendix 1

Taxonomic list suggested for preliminary sorting

Porifera

Cnidaria

Brachiopoda

Mollusca

  Cephalopoda

  other Mollusca

Annelida

Crustacea

  Decapoda

  other Crustacea

Pycnogonida

Echinodermata

  separate all the different groups

Tunicata

Fishes

Appendix 2

Suggested classification for the general arrangement of the reference collection

PROTOZOA

- Foraminifera
- Radiolaria

PORIFERA

- Calcarea
- Hexactinellida
- Demospongiae

CNIDARIA

- Hydrozoa
- Scyphozoa
- Anthozoa

CTENOPHORA

PLATYHELMINTHES

- Turbellaria
- Trematoda
- Cestoda

NEMERTINA

ASCHELMINTHES

- Rotifera
- Priapulida
- Nematoda

ENTOPROCTA

ECTOPROCTA

BRACHIOPODA

MOLLUSCA

- Polyplacophora
- Aplacophora
- Gastropoda
- Scaphopoda
- Pelecypoda
- Cephalopoda

SIPUNCULOIDEA

ECHIUROIDEA

ANNELIDA

- Polychaeta
- Oligochaeta
- Hirudinae

CRUSTACEA

- Branchiopoda
- Ostracoda
- Copepoda
- Mystacocarida
- Branchiura
- Cirripedia
- Malacostraca
  - Leptostraca
  - Syncarida
  - Peracarida
    - Mysidacea
    - Cumacea
    - Tanaidacea
    - Gnathiidea
    - Isopoda
    - Amphipoda
  - Stomatopoda
  - Euphausiacea
  - Decapoda

PYCNOGONIDA

CHAETOGNATHA

POGONOPHORA

ECHINODERMATA

- Crinoidea
- Holothuroidea
- Echinoidea
- Asteroidea
- Ophiuroidea

HEMICHORDATA

TUNICATA

CEPHALOCHORDATA

CYCLOSTOMATA

SELACHII

TELEOSTEI

Clupeiformes
Scopeliformes
Cetomimiformes
Saccopharyngiformes
Anguilliformes
Halosauriformes
Notacanthiformes
Beloniformes
Cyprinodontiformes
Syngnathiformes
Gasterosteiformes
Gadiformes
Lampridiformes
Beryciformes
Zeiformes
Perciformes
Atheriniformes
Scorpaeniformes
Pleuronectiformes
Echeneiformes
Tetraodontiformes
Gobiesociformes
Batrachoidiformes
Lophiiformes

Appendix 3

Basic bibliographic references for determination of animals

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Appendix 4

## List of marine laboratories in Mediterranean and main Museums of the world

Algeria: Centre de Recherches océanographiques
et des Pêches, Jetée Nord
B.P. 90 ALGER-BOURSE

Denmark: Zoological Museum (ZMUC)
Universitetsparken 15
DK 2100 COPENHAGEN

Egypt: Department of Zoology
Cairo University
CAIRO

Institute of Oceanography and Fisheries
Kayet Bay
ALEXANDRIA

France: Muséum national d'Histoire naturelle (MNHN)
57, rue Cuvier
75231 PARIS Cedex 05

Station marine d'Endoume
rue de la Batterie des Lions
13007 MARSEILLE

Université P. et M. Curie
Station zoologique
06230 VILLEFRANCHE SUR MER

Université P. et M. Curie
Laboratoire Arago
66650 BANYULS SUR MER

Musée Océanographique (MOM)
MONACO-Ville
Principauté de Monaco

Germany (Democratic Republic):
Zoologisches Museum von der Humboldt Universität (ZMHU)
Invalidenstrasse 43
DDR-104 BERLIN

Germany (Federal Republic):
Zoologisches Institut und Museum der Universität (ZMH)
Martin Luther King Platz 3
D-2000 HAMBURG 13

Senckenbergische Naturforschende Gesellschaft (SMF)
Senckenberganlage 25
D-6 FRANKFURT am MAIN 1

Greece: Institute of Oceanography and Fisheries Research
Agios Kosmas-Ellinikon
ATHENES

Holland: Zoologisch Museum Universiteit van Amsterdam
Plantage Middenlaan 53
AMSTERDAM - C

Rijksmuseum van Naturlijke Historie (RMNH)
Postbus 9517
2300 LEIDEN RA

Ireland: National museum of Ireland (NMI)
Kildare Street,
DUBLIN 2

Israel: Israel Oceanographic and Limnological Research Ltd
P.O.B. 8030
HAIFA

Department of Zoology
Hebrew University of Jerusalem
JERUSALEM

Italy: Museo Civico di Storia naturale (MSNG)
Via Brigata Liguria 9
16121 GENOVA

Stazione zoologica
Villa Comunale
80121 NAPOLI

Instituto di Zoologia e Anatomia comparata
via Alfonso 32
TRIESTE

Libyan Arab Jamahiriya
Marine Fisheries Center
Bab Elbahr
TRIPOLI

Lebanon: Centre de recherches marines
P.O.B. 11-8281
BEYROUTH

Morocco: Institut scientifique des Pêches maritimes
rue de Tiznit
CASABLANCA

Faculté des Sciences
Université Mohamed V
Avenue Ibn Battouta
B.P. 1014
RABAT

Norway: Zoologisk Museum Universitetet i Bergen (UBNM)
Museplass 3
BERGEN N-5014

Zoologisk Museum (ZMO)
Sarsgt. 1
OSLO 5

Portugal: Museu Bocage (MB)
Rua de Escola Politecnica
LISBOA 2

Spain: Instituto de Investigaciones Pesqueras
Paseo Nacional, s/n
BARCELONA 3

Instituto municipal Ciencias naturales
Museo de Zoclogia
Parque de la Cindadela
Apartado de Correos 593
BARCELONA 3

Instituto Espanol de Oceanografia
c/ Alcala 27, 4°
MADRID 14

Laboratorio oceanografico
Calle Marques della Cenia 119
PALMA DE MAJORCA

Tunisia: Institut nacional scientifique et
technique d'océanographie et de Pêche (INSTOP)
SALAMMBO

United Kingdom:
British Museum (Natural History) (BMNH)
Cromwell Road
LONDON SW7 5BD

Institute of Oceanographic Sciences (IOS)
WORMLEY, Godalming
Surrey GU8 5UB

U.S.A.: U.S. National Museum (USNM)
The Smithsonian Institution
WASHINGTON D.C. 20560

The American Museum of natural History (AMNH)
Central Park West at 79th Street
NEW YORK 10024

California Academy of Sciences (CAS)
Golden Gate Park
SAN FRANCISCO CA 94118

Museum of Comparative Zoology (MCZ)
Harvard University
CAMBRIDGE Massachusetts 02138

U.S.S.R.: Zoological Institute (ZIN)
Academy of Sciences
LENINGRAD 199164

All-Union Research Institute of Marine Fisheries and Oceanography
(V.N.I.R.O.)
17, Krasnoselskaya 17
MOSCOW 107140

Yugoslavia: Institut za oceanografiju i ribarstvo
P.O. Box 114
58000 SPLIT

Centar za istrazivanje mora
52210 ROVINJ

Appendix 5

## Useful addresses

## (Manufacturers' and suppliers' addresses)

- PAPER:

| | |
|---|---|
| Goatskin parchment, blue white 160g/m$^2$ : | Wiggins Teape Paper Ltd.,<br>Park House,<br>165-177 The Boradway,<br>Wimbledon SW191NR<br>ENGLAND |
| Papertex paper: | IPA Departement, Spari-France<br>47 rue de la Chapelle<br>75018 PARIS<br>FRANCE |
| Tyvek paper: | Papeteries de Guyenne<br>46, Quai des Célestins<br>75004 PARIS<br>FRANCE |

- MACHINE FOR PERFORATING LABELS

Bernard Prin et Cie
20-24 rue de la Vanne
92120 Montrouge
FRANCE

- RAPID LABELLING OF INDIVIDUAL FISH:

| | |
|---|---|
| Swift gun and tags: | Sofadi,<br>43 rue de Stalingrad<br>93100 Montreuil sous bois<br>FRANCE |

- STORAGE JARS

| | |
|---|---|
| Wide range of jars with snap-on polythene lids from 70 to 2500 ml (particularly valuable for temporary storage): | Kay R. Grathwol,<br>Dronningens Tvaergade 35,<br>1302 KOBENHAVN K,<br>Danemark |
| Wide range of jars with quick release lids from 250 to 3000 ml (particularly valuable for long term storage) and wide range of small vials with plastic caps: | Les Verreries Françaises<br>4, rue de la Paix<br>75002 PARIS<br>FRANCE |
| Very wide range of glass with lids in shape of glass discs: | Glas-Keramik<br>Kronenstrasse 19/19a<br>108 Berlin, D.D.R. |

- PLASTIC STORAGE TUBS AND CONTAINERS

| | |
|---|---|
| Many sizes of polythene tubs with sturdy lids.<br>The following sizes are particularly useful:<br>10 and 15 litres (circular)<br>25 litres (square)<br>75 litres (circular) | W.C.B. Containers Ltd.,<br>Stanford Works,<br>Bayley Street<br>Stalybridge<br>Cheshire SK15 1QQ<br>ENGLAND |
| Circular plastic tubs, with metal carrying handles, with capacities of 5, 12,5 and 15 litres: | Barclay Stuart Plastics Ltd.,<br>Brunswick Street,<br>Luton, Bedfordshire, ENGLAND |
| Many models and sizes of containers from 10 to 1200 litres | Gilbert Lacroix (Gilac)<br>2, rue Marengo<br>75001 PARIS<br>FRANCE |
| Wide range of sizes of "box ware" with plastic lids | Société A.L. Guillouard<br>15, Boulevard des Martyrs Nantais<br>44000 NANTES, FRANCE |
| Barrel-shaped containers | Sté Feralco<br>9, rue St Sébastien<br>75011 PARIS<br>FRANCE |

- SIEVES

Endecotts (Test Sieves)Ltd.
Lombard Road
Marden Factory Estate,
London SW 19,
England

- CHEMICALS

| | |
|---|---|
| MS 222: | Sandoz S.A.<br>Division: Produits pour la chimie pharmaceutique et alimentaire<br>CH-4002 Bâle<br>SUISSE |
| All chemicals: | Prolabo<br>12, rue Pelée<br>75011 Paris<br>France<br><br>BDH Chemicals Ltd.<br>Poole<br>Dorset BH 12 4NN<br>England |
| Propylene phenoxetol: | NIPA Laboratories Ltd.,<br>Treforest, Glamorganshire<br>Wales, U.K. |

- BOLTING SILK

John Stanier and Co.,
Silk Merchants
Manchester Wire Works
Manchester
England

- NYLON MESH

Henry Simon Ltd.,
Cheadle Heath,
Stockport, Cheshire
ENGLAND

- COLLECTING EQUIPMENT (PLANKTON NETS, DREDGES, TRAWLS) OCEANOGRAPHIC DEVICES:

General equipment:

Marine Biological Association of the U.K.
The Laboratory
Citadel Hill
Plymouth
Devon
ENGLAND

Benthos Inc.
North Falmouth,
Mass. 02556
U.S.A.

EGMO
Bd Manfille
Port de Commerce
29000 Brest
FRANCE

Marinovitch Trawl Co, Inc.
1317 East First street
Biloxi, Mississippi 39533
USA

Markdan
Egeallé
DK 8600 Silkeborg
Denmark

Hydro-Bios Aparate Bau GMBH,
23, Kiel-Holtenau
W. Germany

Mecabolier
57, avenue de la République
94290 Villeneuve le Roi
FRANCE

Rigosha Co. Ltd.,
3 Kajicho 1-Chome Kandai
Chiyoda-ku
Tokyo
JAPON

Fishing nets and twine:

Ets Le Drezen
Léchiagat
29 115 LE GUILVINEC
B.P. 46
France

Ets Kersaudy et le Meur
Z.I. de Ploudaniel
29 174 DOUARNENEZ
France

Bridport-Gundry Ltd. (Beetons Branch),
Bettery Green Road,
Lowestoft,
Suffolk
England

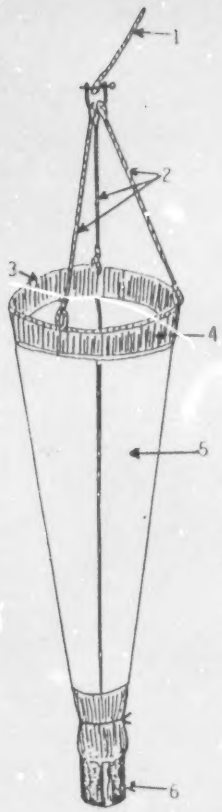



Fig. 1 - Standard plankton townet (redrawn from Newell and Newell, 1973)
1. warp
2. bridles
3. ring
4. canevas collar
5. net
6. bucket



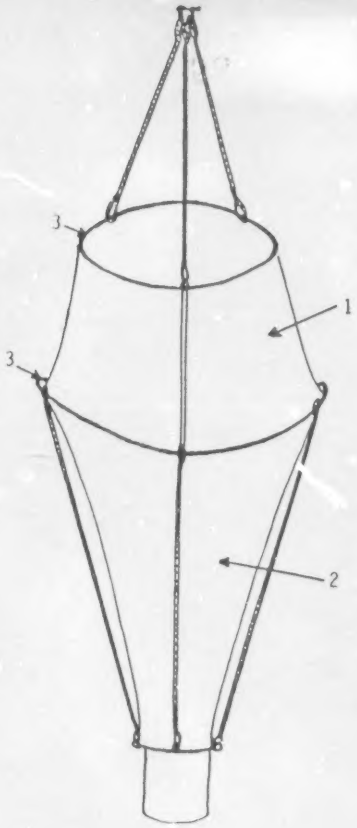



Fig. 2 - Hensen net (r drawn from Newell and Newell, 1973)
1. canevas collar
2. net
3. ring

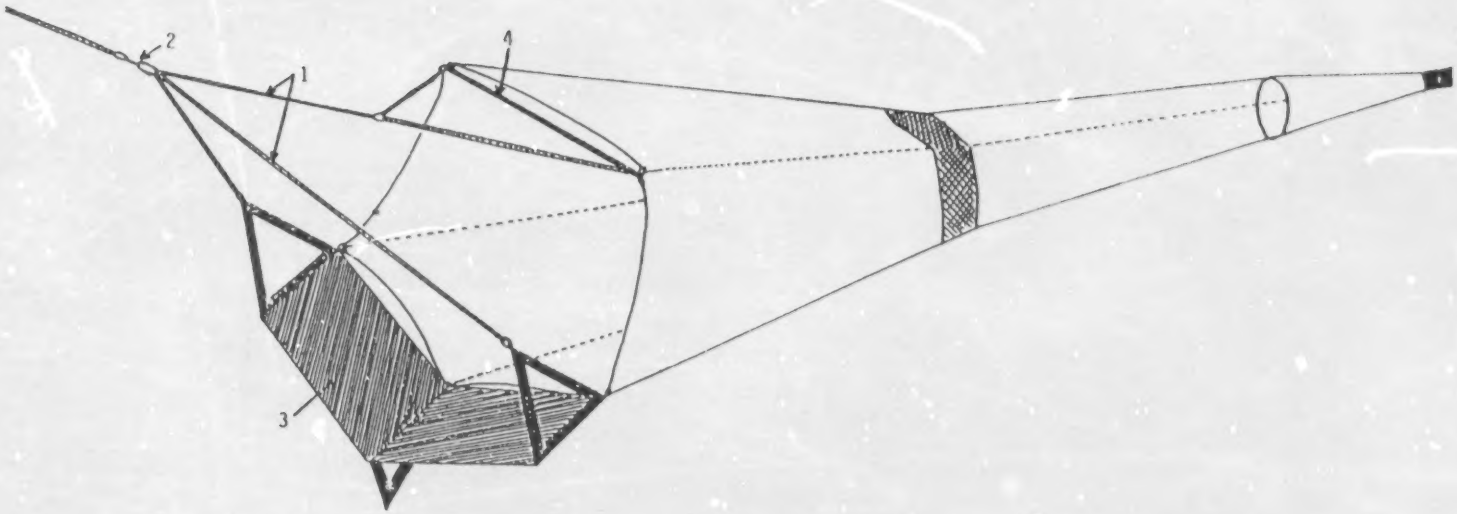



Fig. 3 - Isaacs-Kidd midwater trawl
1. bridle
2. swivel
3. depressor
4. spreader-bar

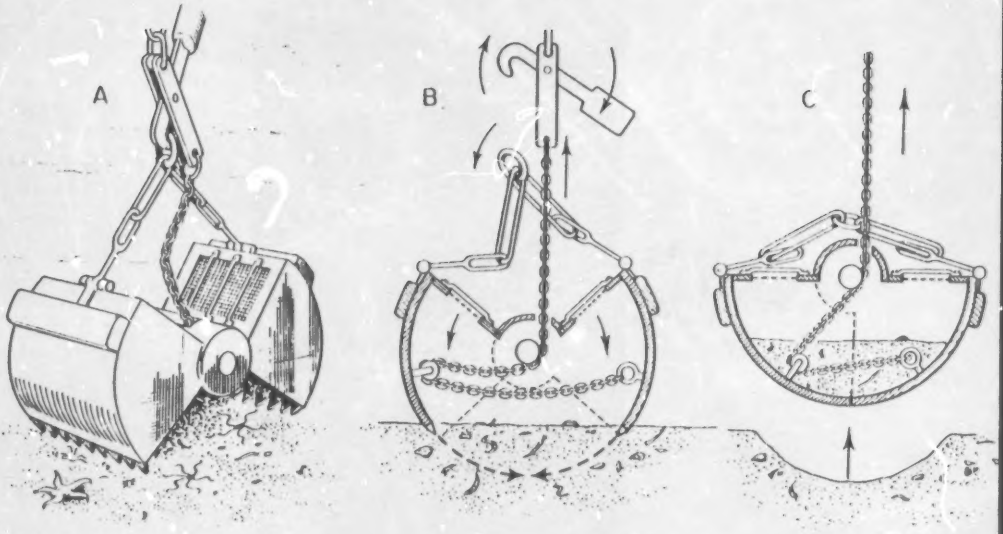



Fig. 4 - Petersen grab taking a sample from the sea bed (reproduced from Holme, 1964)

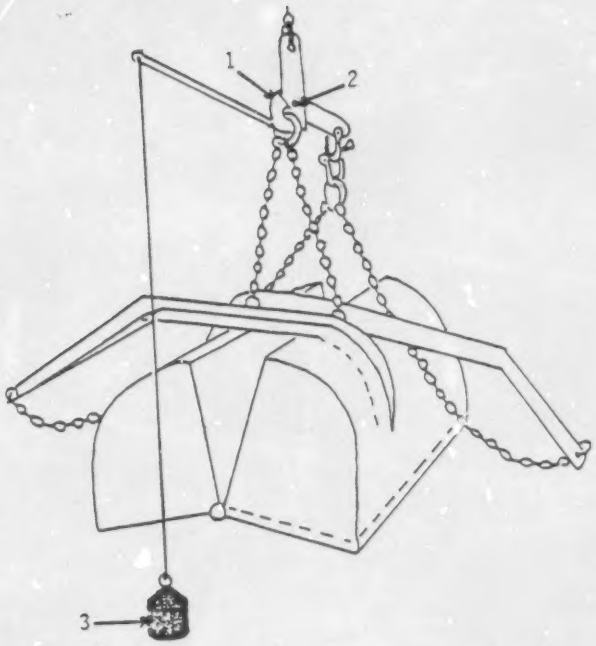



Fig. 5 - Van Veen grab (redrawn from Lassig, 1965)
1. Release hook
2. hole for locking pin
3. pilot weight

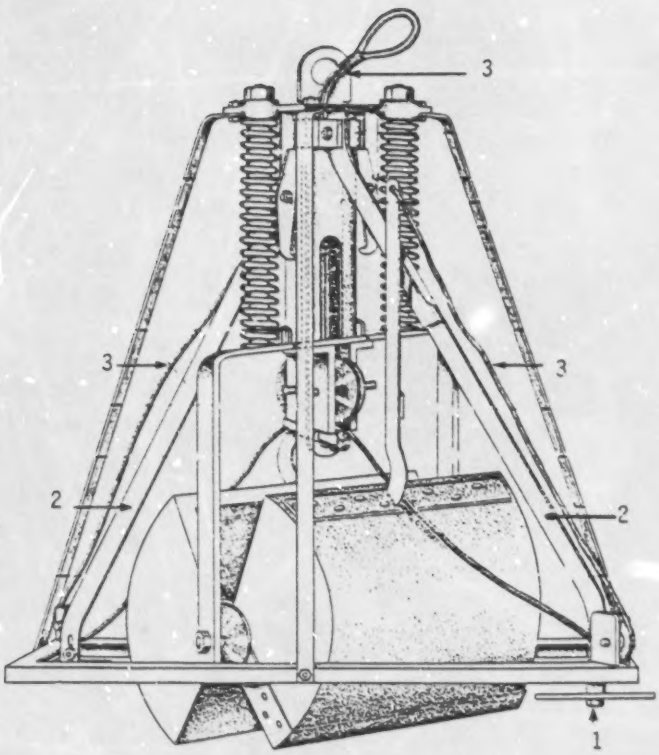



Fig. 6 - Smith and McIntyre grab (from Holme, 1964)
1. trigger plate
2. release bar
3. closing wire

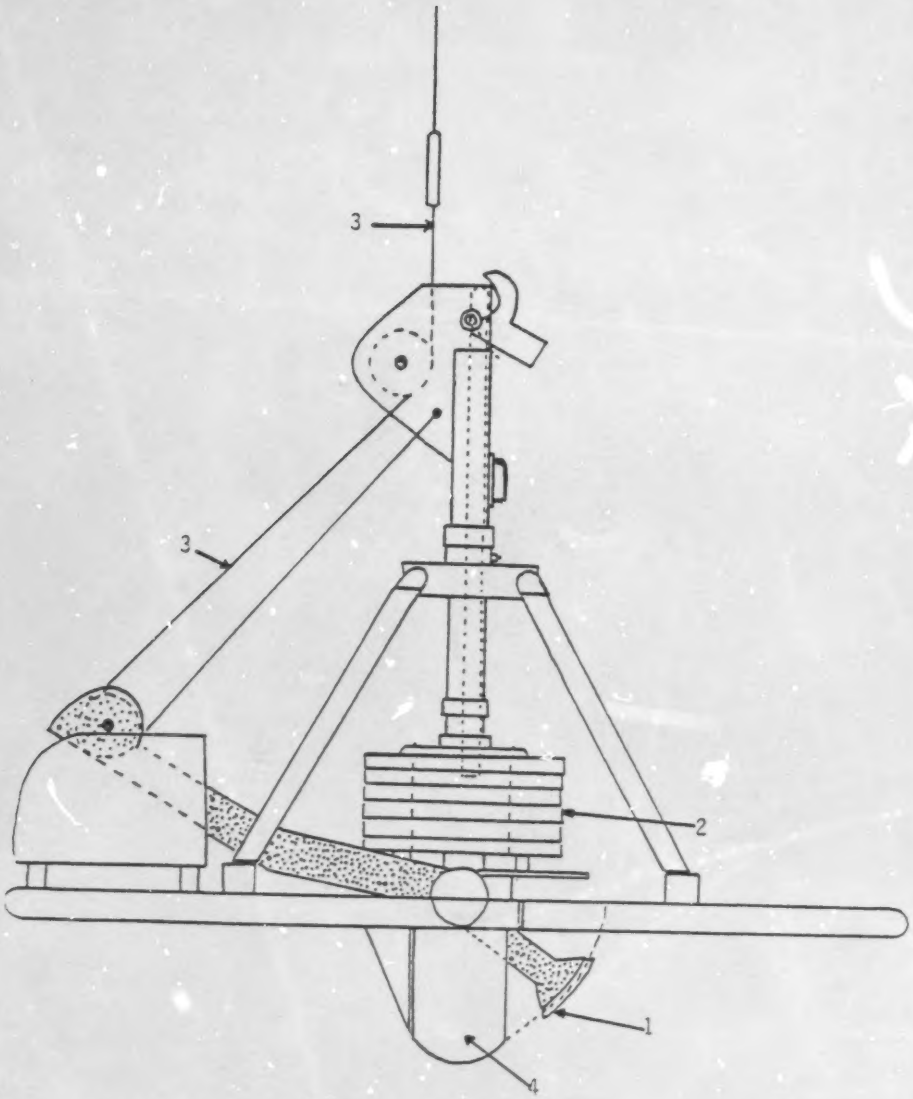



Fig. 7 - Reineck box corer (redrawn from Holme, 1971)
1. knife
2. variable weights
3. wire
4. box

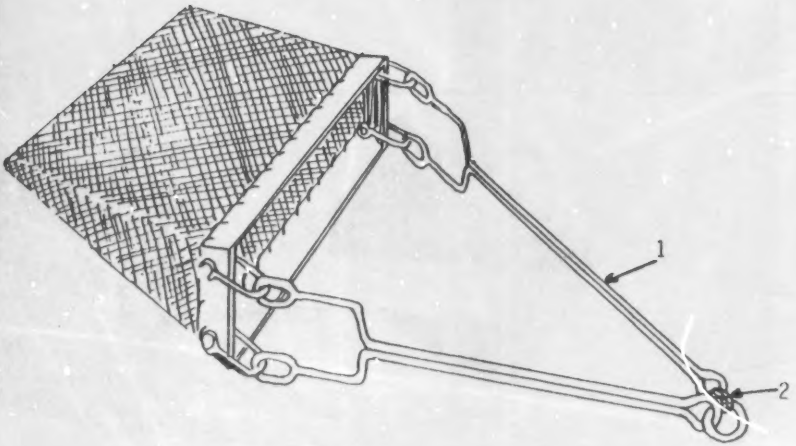



Fig. 8 - Naturalist's dredge (redrawn from Holme and McIntyre, 1971)
1. towing arms
2. weak link

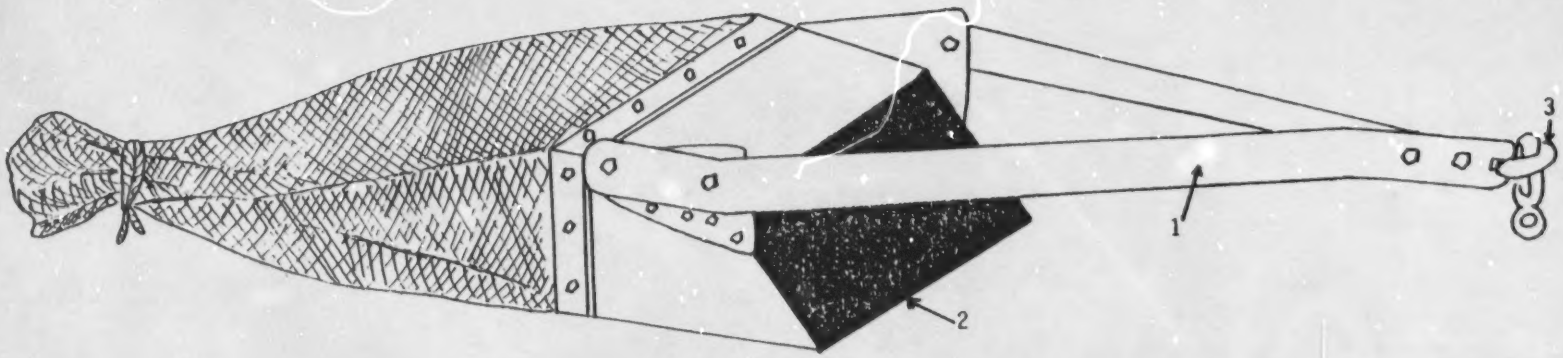



Fig. 9 – Anchor dredge
1. towing arms
2. scoop
3. towing eye

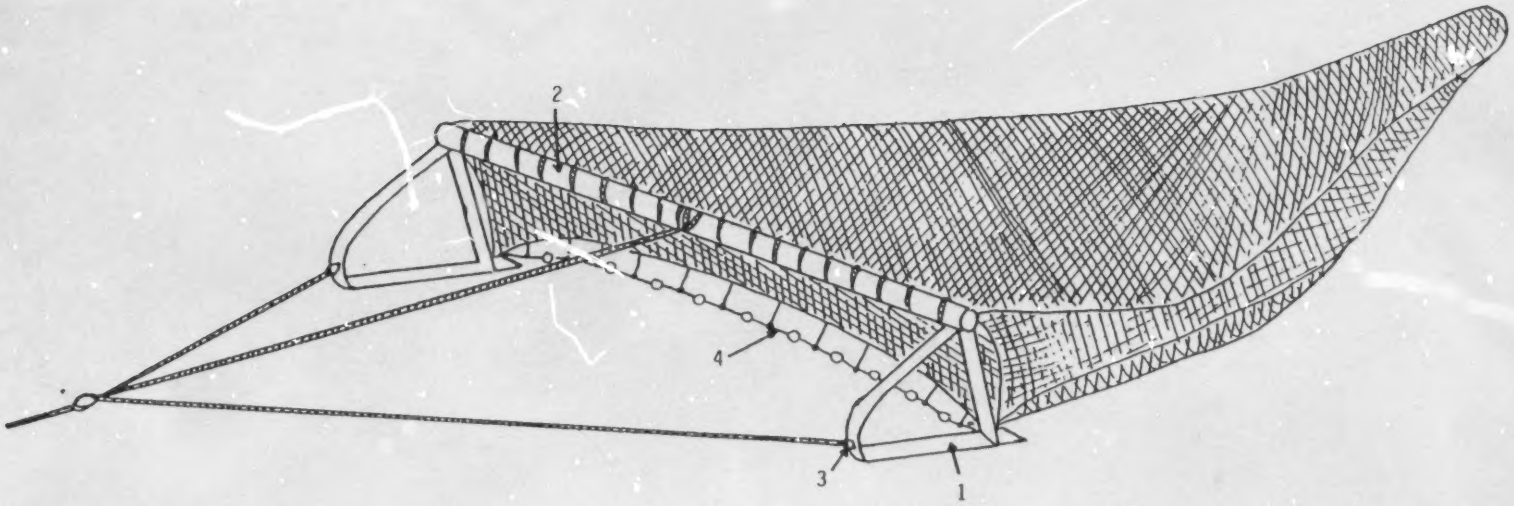



Fig. 10 - Beam trawl
1. skid
2. beam
3. towing eyes
4. tickler-chain

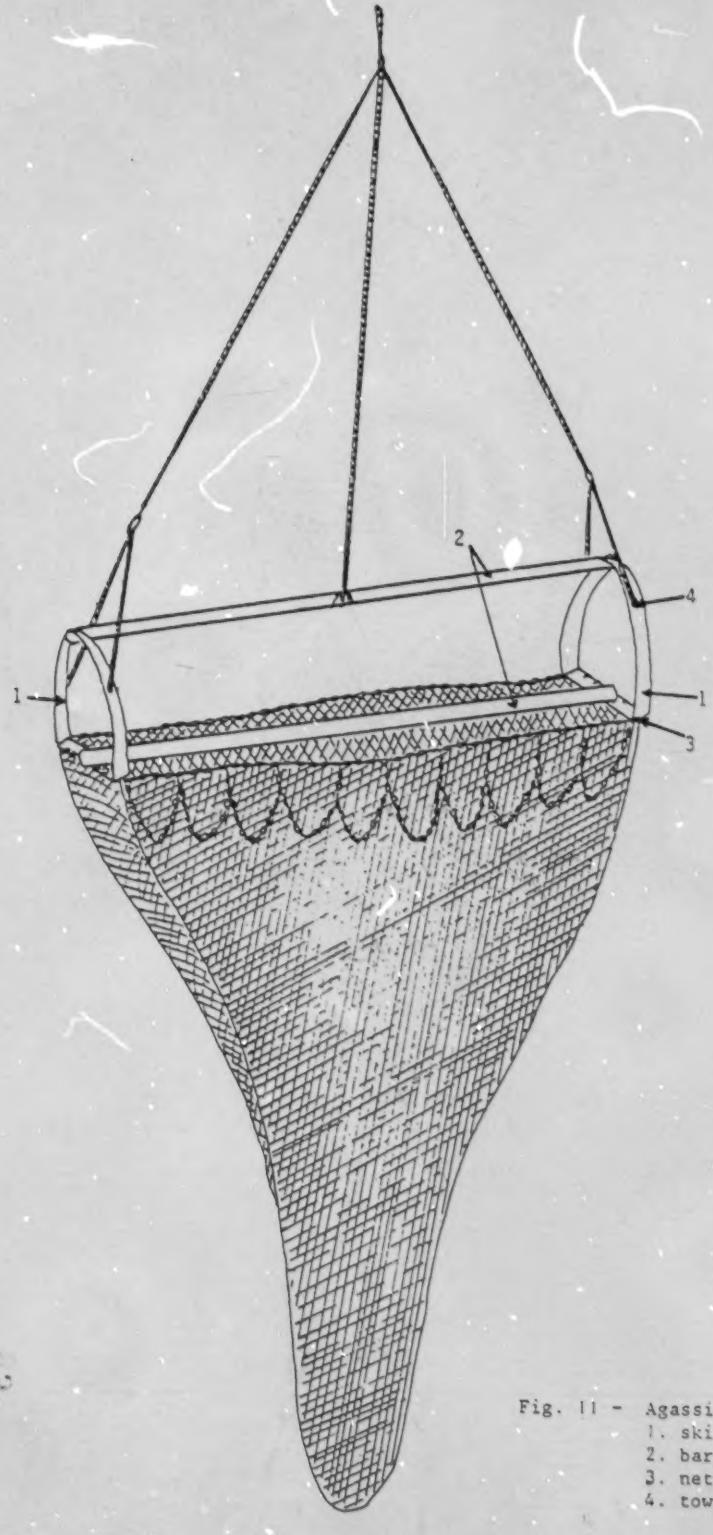
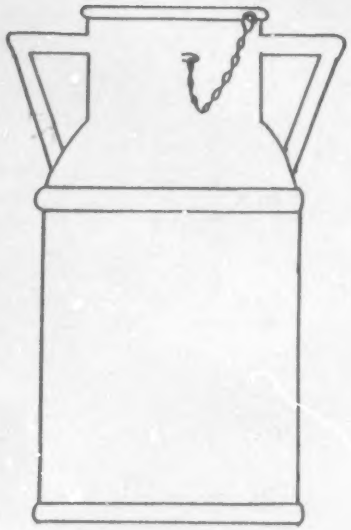



Fig. 11 - Agassiz trawl
1. skid
2. bars
3. net attachement eyes
4. towing eye



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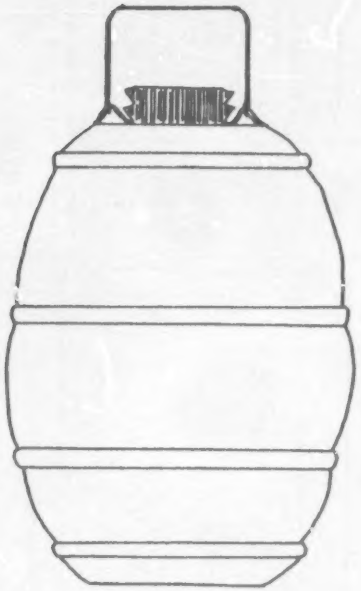

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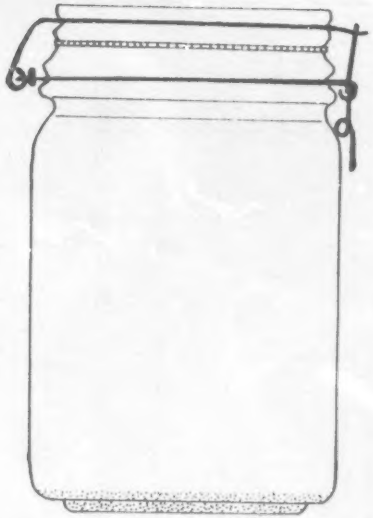
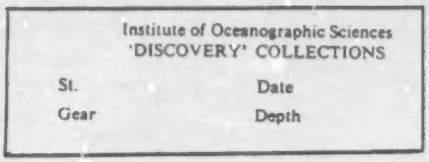
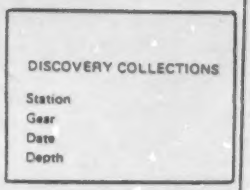
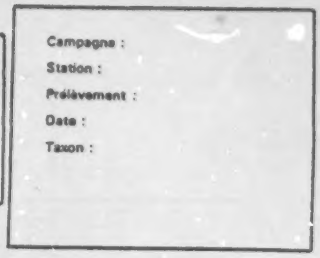


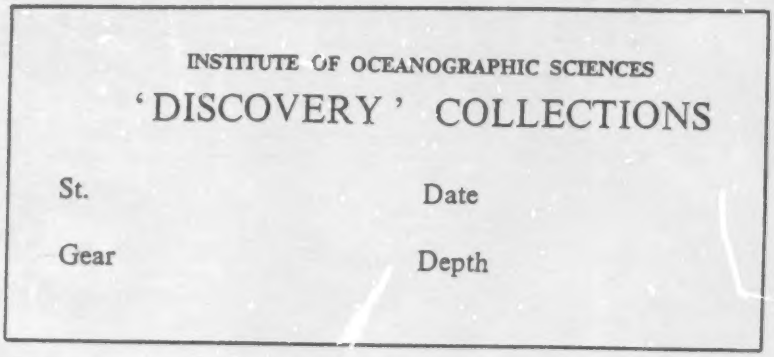
1

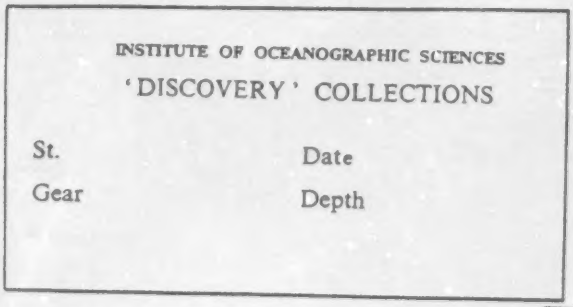
Fig. 12 - Different types of jars and containers
1. glass Jar with quick release lid
2. milk-churn
3. plastic barrel

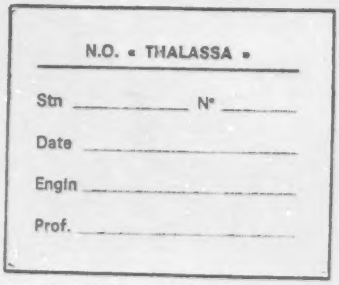


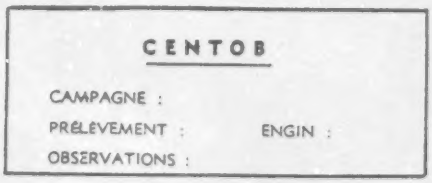



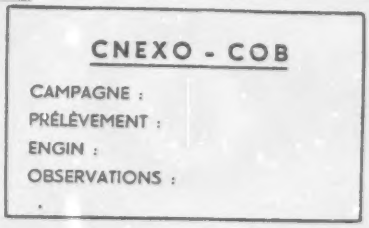




Fig. 13 - Examples of pre-printed labels

| BATIMENT | CAMPAGNE | DATE | LIEU |
|---|---|---|---|
| | | | |

| STATION N° |
|---|
| PRELEVEMENT N° |

*Type d'Engin* _____

*Lest* Kg

_____ *Vitesse* Nœuds

*Engin au fond* h mn *Sonde* *Position* φ =
G =

*Ligne filée*

*Observations (variation vitesse, virage-dévirage câble, etc...* _____

_____

_____

_____

_____

_____

*Départ du fond* h mn *Sonde* *Position* φ =
G =

_____

*Nature et topographie du fond* _____

_____

*Prélèvement (observations générales sur la faune, flore, le sédiment, le volume recueilli...)*

_____

_____

_____

_____

*Observations (bon ou mauvais travail de l'engin, incidents...)*

_____

_____

_____

_____

CHEF DE QUART

Fig. 14 - Station sheet

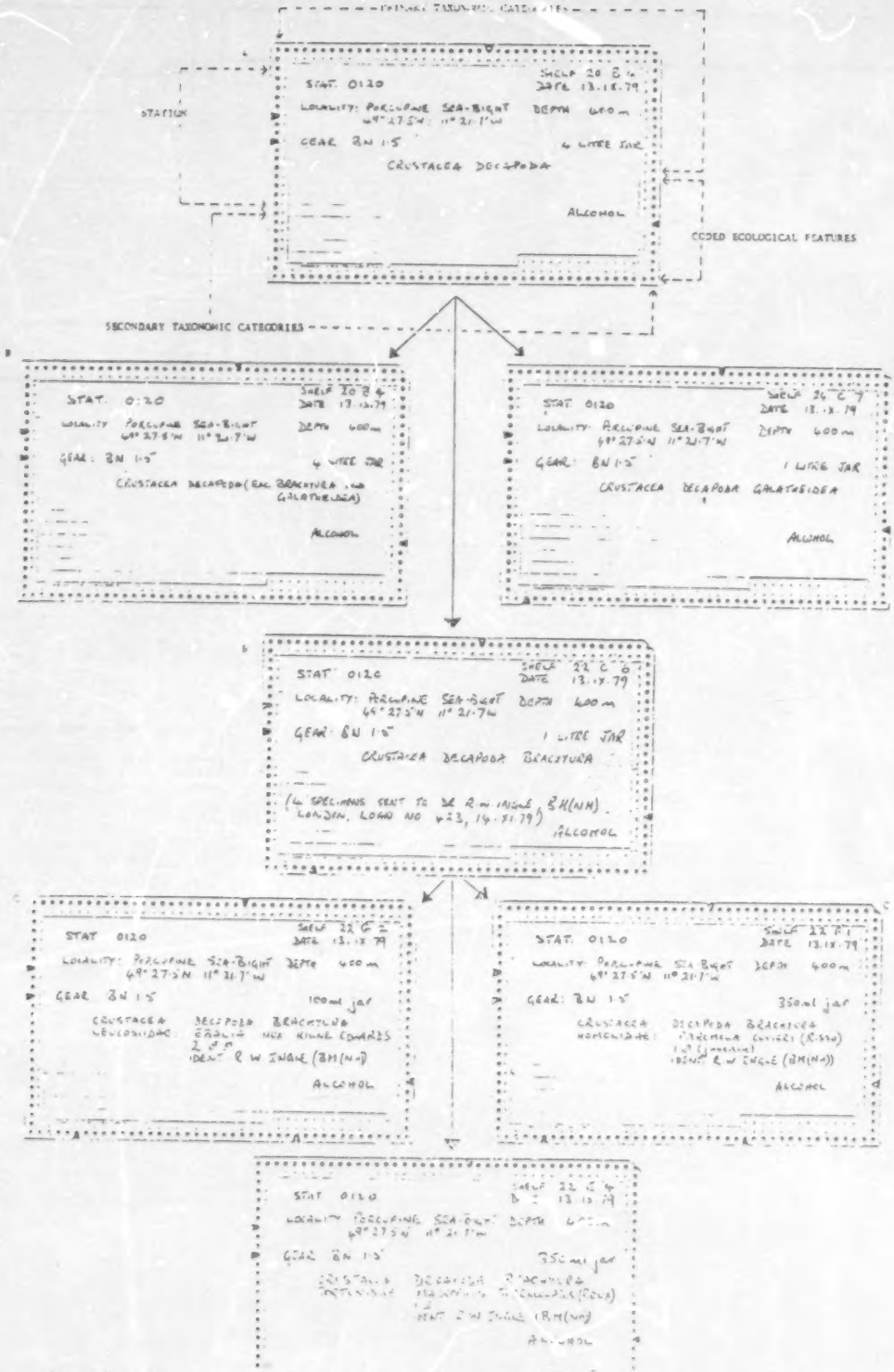



Fig. 15 - Suggested documentation system using edge-punched filing cards, with an example of the cards successively produced during sorting.

MUSÉUM NATIONAL D'HISTOIRE NATURELLE

Page

ICHTYOLOGIE GÉNERALE ET APPLIQUEE

43, RUE CUVIER
75231 PARIS CEDEX 05
TELEPHONE 331-40-10

## BORDEREAU D'ENVOI/INVOICE OF SPECIMENS N° 26

Destinataire / To : Institute of Oceanographic Sciences (Mr A. RICE)
Wormley
Godalming
Surrey GU8 5UB
Angleterre

Envoyé par / Sent by : J.C. Hureau  Signature : J.C. Hureau

Date de l'envoi / Sent on : 26 novembre 1979

Nombre de paquets / Number of packages : 1

Mode d'expédition / Method of shipment : poste

Recommandé / Registered : oui

Prêt / Loan ☒  Don / Gift ☐  Échange / Exchange ☐  Retour de matériel / Return of material ☐

| N° DE COLLECTION/ COLLECTION NUMBER | NOMBRE DE SPÉCIMENS/ NUMBER OF SPECIMENS | NOM/ NAME | INFORMATIONS/ DATA |
|---|---|---|---|
| MNHN 1979-10 | 5 | Sparus aurata | coll. Biaçores "Jean Charcot" |
| MNHN 1979-325 | 2 | Nototheniidae | coll. MD 08 (à déterminer) |
| MNHN 1975-203 | 15 | Brama sp. | coll. Duhamel (à déterminer) |
| MNHN B 849 | 1 | Muraenophis saga Risso, 1810 | Holotype |

MNHN, Paris - 750539 - 2000 - 2.79

A la réception des spécimens, prière de signer et renvoyer la feuille blanche/
Upon receipt of specimens, please sign and return the white copy of this invoice

Reçu en bon état / Received in good order ☐  Sauf mention / Except as noted ☐

Date de réception / Date of receipt

Nom / Name :

S'il s'agit d'un prêt, à renvoyer avant le/

Signature :

Fig. 16 - Example of a completed loan form